Endotoxin priming of the cyclooxygenase-2-thromboxane axis in isolated rat lungs

M. ERMERT, M. MERKLE, R. MOOTZ, F. GRIMMINGER, W. SEEGER, AND L. ERMERT

1Institute of Anatomy and Cell Biology, 2Department of Internal Medicine, and 3Department of Pathology, Justus-Liebig-University Giessen, 35385 Giessen, Germany

Am J Physiol Lung Cell Mol Physiol 278: L1195–L1203, 2000.—Enhanced prostanoïd generation has been implicated in vascular abnormalities occurring during endotoxemia and sepsis, and the lung is particularly prone to such events. Prostanoids are generated from arachidonic acid (AA) via cyclooxygenase (COX)-1 or -2, both isoenzymes recently demonstrated to be expressed in different lung cell types. Upregulation of COX may underlie the phenomenon that endotoxin (lipopolysaccharide [LPS])-exposed lungs show markedly enhanced vasoconstrictor responses to secondarily applied stimuli (priming). Isolated rat lungs were perfused with a physiological salt buffer solution in the absence and presence of 1.5% rat plasma and exposed to different concentrations of LPS (1,000 or 10,000 ng/ml) during a 2-h priming period. No change in physiological variables was noted during this period, although enhanced baseline liberation of both thromboxane (Tx) A2 and prostacyclin (PGI2) as well as of tumor necrosis factor (TNF)-α was evident compared with that in control lungs in the absence of LPS. LPS priming caused a significant elevation in AA-induced pulmonary arterial pressure, ventilation pressure, and lung weight gain. Concomitant increased levels of TxA2 were found in the buffer perfusate. All changes were largely suppressed by three selective, structurally unrelated COX-2 inhibitors (NS-398, DUP-697, and SC-236) in both buffer- and buffer-plasma-perfused lungs. Anti-TNF-α neutralizing antibodies were ineffective under conditions of buffer perfusion. In the presence of plasma components, manifold augmented TNF-α generation was noted, and anti-TNF-α antibodies significantly suppressed the increase in ventilation pressure but not in the vascular pressor response and lung edema formation. We conclude that the propensity of LPS-primed lungs to respond with enhanced vasoconstriction, edema formation, and bronchoconstriction to a secondarily applied stimulus proceeds nearly exclusively via COX-2 and increased Tx generation, with TNF-α generation being involved in the change in bronchomotor reactivity in the presence of plasma constituents. In context with recent immunohistological investigations, LPS-induced upregulation of the COX-2-thromboxane synthase axis in vascular and bronchial smooth muscle cells is suggested to underlie these events.

lipopolysaccharide; isolated perfused rat lung; tumor necrosis factor; selective cyclooxygenase-2 inhibition

ENDOTOXEMIA-INDUCED pathophysiological changes are thought to contribute largely to circulatory abnormalities and organ failure under conditions of sepsis, with persistently high mortality rates in critically ill patients (5, 41). Among the organs affected under these conditions, acute lung injury [acute respiratory distress syndrome (ARDS)] is of major importance (5, 27). Endotoxin, a lipopolysaccharide (LPS) from gram-negative bacteria, is known to provoke proinflammatory cytokine synthesis similar to that of tumor necrosis factor (TNF)-α and enhanced generation of eicosanoids. Among the latter, prostaglandins (PGs) and thromboxane (Tx), which have been implicated in pulmonary vascular abnormalities and changes in bronchomotor tone in acute lung injury (38, 40), are generated by conversion of arachidonic acid (AA) to the unstable intermediate PGG2 / PGH2, catalyzed by two cyclooxygenase (COX) isoenzymes. Initially, COX-1 was noted to be constitutively expressed in various organ systems, being involved in the regulation of physiological functions, whereas COX-2 was assumed to be quiescent under normal physiological conditions, being upregulated only during inflammatory circumstances, then providing the enzymatic basis for proinflammatory prostanoïd effects. Meanwhile, however, both COX isoenzymes have been shown to occur constitutively in several organ systems (18, 21, 26, 33).

Concerning the lung architecture, Ermer et al. (14) recently showed COX-2 to be constitutively expressed in various pulmonary cell types including bronchial epithelial cells, bronchial smooth muscle cells, macrophages, mast cell-like cells, and smooth muscle cells of partially muscular vessels, which are operative as pulmonary resistance vessels (31). Moreover, an immunostaining study (13) also demonstrated the presence of thromboxane synthase in the smooth muscle cells of the partially muscular vessels in noninflamed lungs. In contrast, no COX-2 or thromboxane synthase immunostaining was noted in endothelial cells under baseline conditions. In accordance with these morphological data, a recent pharmacological study (11) indeed demonstrated that vasoconstrictor responses to AA in noninflamed lungs, proceeding via Tx generation that supervenes the simultaneously enhanced prostacyclin formation, are operated largely via COX-2 and not via COX-1 activity.

Lung reactivity to vasoactive stimuli is known to be altered under inflammatory conditions. This was impressively demonstrated for endotoxin under experi-
MATERIALS AND METHODS

Reagents. AA and acetylsalicylic acid were obtained from Paesel and Lori (Frankfurt, Germany). LPS (Salmonella abortus equi, S-form) was purchased from Cytogen (Bodenheim, Germany). All other biochemicals were obtained from Merck (Darmstadt, Germany). ELISA kits for the determination of 6-keto-PGF1α and TxB2 were obtained from Cayman Chemical (Ann Arbor, MI). The TNF-α ELISA kit was supplied by BioSource (Deerfield, IL). An anti-TNF-α neutralizing antibody (ICC-TNF-9A) was obtained from Ic Chemikalien (Ismaning, Germany). The COX-2 inhibitor NS-398 was purchased from BIOMOL (Hamburg, Germany). The COX-2 inhibitor DUP-697 was kindly provided by DuPont Pharmaceuticals (Wilmington, DE), and the COX-2 inhibitor SC-236 was a gift from Searle (St. Louis, MO).

Animals. CD rats (Sprague-Dawley) were obtained from Charles River (Sulzfeld, Germany). All experimental procedures were performed in conformity with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals [DHHS Publication (NIH) No. 86-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892].

Lung isolation and perfusion. The rats (male, body weight 350–400 g) were deeply anesthetized with pentobarbital sodium (100 mg/kg body wt ip). After local anesthesia with 2% xylocaine and a median incision, the trachea was dissected and a tracheal cannula (Hugo Sachs Elektronik, March-Hugstetten, Germany) was immediately inserted. A median laparotomy was performed, and the rats were anticoagulated with 1,000 U of heparin. Subsequently, mechanical ventilation was started with 4% CO2, 17% O2, and 79% N2 (tidal volume 4 ml, frequency 65 breaths/min, end-expiratory pressure 3 cmH2O) with a small-animal respirator KTR-4 (Hugo Sachs Elektronik). After a midsternal thoracotomy, the right ventricle was incised, a cannula (2.0-mm diameter; Hugo Sachs Elektronik) was fixed in the pulmonary artery, and the apex of the heart was cut off to allow pulmonary venous outflow. Simultaneously, pulsatile perfusion with the buffer solution was started. The buffer contained 2.4 mM CaCl2, 1.3 mM MgCl2, 4.3 mM KCl, 1.1 mM KH2PO4, 125.0 mM NaCl, and 25 mM NaHCO3 as well as 13.32 mM glucose (pH ranged between 7.35 and 7.40).

The lungs were carefully excised, with any damage being avoided, while being perfused with the buffer solution and placed in an upright position. Next, a cannula (Hugo Sachs Elektronik) was fixed in the left atrium through the left ventricle to obtain a closed perfusion circuit.

After extensive rinsing of the vascular bed, the lungs were recirculatingly perfused with a pulsatile flow of 13 ml/min. The alternate use of two separate perfusion circuits, each containing 100 ml, allowed the repetitive exchange of perfusion fluid. Perfusion pressure, ventilation pressure (VP), and weight of the isolated organ were registered continuously (Combitrans Monitoring Set Med. II for arterial blood pressure measurement, Braun Melsungen, Germany; transducer, Marquette Hellige, Neudrossenfeld, Germany). The left atrial pressure was set at 2 mmHg under baseline conditions (0 referenced at the hilum) to guarantee zone III conditions at end expiration throughout the lung.

Lungs selected for the study were those that 1) had a homogeneous white appearance without signs of hemostasis or edema formation, 2) had pulmonary arterial pressure (PAP) and VP in the normal range (PAP 5–7 mmHg, VP 4–5 mmHg), and 3) were isogravimetric during a steady-state period of 30 min.

Experimental protocol. All experiments were undertaken both in buffer-perfused lungs and in lungs perfused with an admixture of 1.5% rat plasma in the buffer fluid. In total, 140 isolated lung experiments were performed. Control lungs were perfused with recirculating fluid under standard conditions for 125 min without any drug application (n = 5 experiments for each buffer and buffer-plasma perfusion). In experiments without LPS application (n = 5 each), free AA was admixed to the perfusate at a concentration of 5 μmol/l after 2 h of perfusion.

As shown in Fig. 1, two different dosages of LPS were admixed to the perfusate at the onset of the 2-h perfusion period, resulting in concentrations of 1,000 or 10,000 ng/ml in the recirculating buffer fluid (n = 5 experiments each). At the end of the 2-h perfusion period, 5 μmol/l of AA were administered as in the lungs perfused in the absence of LPS.
The following concentrations and combinations of challenge and inhibitor were used to inhibit selectively COX-2 activity (Fig. 1). Lungs were challenged with either 1,000 or 10,000 ng/ml of LPS; after 110 min of perfusion, NS-398 was administered at a final concentration of either 10 or 25 µmol/l (n = 5 experiments for each combination of LPS dosage and inhibitor dosage). Two additional COX-2-selective inhibitors were applied after preceding high-dose LPS stimulation (10,000 ng/ml). DUP-697 was used at a concentration of 25 µmol/l, and SC-236 was used at a concentration of 10 µmol/l (n = 5 experiments/group). The inhibitors were applied 10 min before AA administration to guarantee a sufficient preincubation period for structural binding and irreversible inhibition of COX-2 (30).

Further experiments were conducted with the application of an anti-TNF-α neutralizing antibody at two different concentrations (125 and 250 µg/l). One microgram of anti-TNF-α antibody is required to neutralize 25 µg of TNF-α in the buffer perfusate (neutralizing capacity). Rat lungs were challenged with 10,000 ng/ml of LPS, and the anti-TNF-α antibody was administered either 5 min before or 60 min after LPS administration (n = 5 experiments for each combination of anti-TNF-α antibody dosage and timing). The perfusate admixture of AA (5 µmol/l) was administered as in the preceding protocols.

In selected experiments, samples for perfusate analysis were taken at the onset of the 2-h perfusion period (0 min), after 1 h of perfusion (60 min), after 2 h of perfusion before application of AA (120 min), and 2 and 5 min after AA admixture application (122 and 125 min, respectively).

Perfusate analysis. TxA2 and PGI2 were assayed by ELISA from the recirculating buffer fluid as their stable hydrolysis products TxB2 and 6-keto-PGF1α, respectively. In addition, TNF-α in the buffer perfusate was measured by the ELISA technique.

Statistical analysis. Analysis of variance followed by the Newman-Keuls post hoc test and Student’s t-test for unpaired data was used to evaluate differences among different groups. A value of P < 0.05 was considered significant. All data are means ± SE.

RESULTS

Buffer-perfused lungs. In control lungs, no weight gain (ΔW) or change in PAP or VP was registered over the entire observation period of 2 h. In the absence of preceding LPS administration, 5 µmol/l of free AA provoked a rapid increase in PAP by >5 mmHg within 5 min (Table 1, Fig. 2). Concomitantly, a ΔW was noted, amounting to >1 g within 5 min. In contrast, VP remained unchanged in response to AA administration.

PAP, VP, and ΔW were within normal ranges over 2 h in lungs perfused with 1,000 or 10,000 ng/ml of LPS. However, a markedly enhanced responsiveness to the subsequent AA challenge was noted. The PAP increase registered 5 min after AA administration was more than doubled, a rapid ΔW of >6–8 g occurred, and the VP increased to approximately twofold baseline values (Table 1, Fig. 2). The response in the presence of 10,000 ng/ml of LPS slightly surpassed that in the presence of 1,000 ng/ml of LPS.

Preapplication of the selective COX-2 inhibitors NS-398 (10 µmol/l), SC-236 (10 µmol/l), or DUP-697 (25 µmol/l) 10 min before the administration of AA suppressed the AA-induced PAP response to near baseline values both in control lungs and in lungs undergoing a preceding 2-h LPS incubation period (Table 2, Fig. 2). In parallel, the AA-induced ΔW and VP, which were

Table 1. ΔPAP, ΔW, and ΔVP in response to AA challenge impact of LPS priming

<table>
<thead>
<tr>
<th>Absence of plasma</th>
<th>ΔPAP, mmHg</th>
<th>ΔW, g</th>
<th>ΔVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, no LPS</td>
<td>5.14 ± 0.67</td>
<td>1.09 ± 0.31</td>
<td>0.17 ± 0.11</td>
</tr>
<tr>
<td>1,000 ng/ml of LPS</td>
<td>10.66 ± 0.77</td>
<td>6.26 ± 1.21</td>
<td>2.93 ± 0.34</td>
</tr>
<tr>
<td>10,000 ng/ml of LPS</td>
<td>12.01 ± 0.63</td>
<td>7.60 ± 0.79</td>
<td>3.14 ± 0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Presence of 1% plasma</th>
<th>ΔPAP, mmHg</th>
<th>ΔW, g</th>
<th>ΔVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, no LPS</td>
<td>0.81 ± 0.15</td>
<td>0.10 ± 0.02</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>1,000 ng/ml of LPS</td>
<td>5.53 ± 0.56</td>
<td>1.31 ± 0.27</td>
<td>1.79 ± 0.24</td>
</tr>
<tr>
<td>10,000 ng/ml of LPS</td>
<td>5.84 ± 0.57</td>
<td>1.34 ± 0.33</td>
<td>2.21 ± 0.26</td>
</tr>
</tbody>
</table>

Values are means ± SE of changes occurring within 5 min of arachidonic acid (AA) application; n = 5 independent experiments/group. ΔPAP, increase in pulmonary arterial pressure; ΔW, weight gain; ΔVP, increase in ventilation pressure; LPS, lipopolysaccharide. Lungs were buffer or buffer-plasma perfused in absence (control) and presence of LPS. AA (5 µmol/l) was admixed to perfuse after a 2-h recirculation period. Significant difference from respective control lungs: *P < 0.05; †P < 0.01; ‡P < 0.001.
prominent only in the LPS-primed lungs, was markedly reduced by all three COX-2 inhibitors (Table 2, Fig. 2). Application of a higher dose of the COX-2 inhibitor NS-398 (25 µmol/l) nearly fully extinguished the PAP increase, ΔW, and increase in VP in response to AA administration in lungs primed with 1,000 or 10,000 ng/ml of LPS (Table 2, Fig. 2).

In buffer-perfused lungs undergoing priming with 10,000 ng/ml of LPS for 2 h, the administration of anti-TNF-α neutralizing antibodies either 5 min before or 60 min after LPS administration did not affect the AA-induced responses of PAP, ΔW, or VP (Table 3, Fig. 3).

In control lungs, only minor amounts of TxB2 and 6-keto-PGF1α were liberated into the recirculating buffer fluid over the entire observation period (all data <50 pg/ml for TxB2 and <750 pg/ml for 6-keto-PGF1α). LPS priming with 1,000 and 10,000 ng/ml of LPS caused a progressive but moderate accumulation of TxB2, amounting to 115 ± 20 and 176 ± 23 pg/ml, respectively, within 2 h. Subsequent administration of AA

Table 2. Impact of COX-2 inhibition in AA-elicited response in LPS-primed lungs

<table>
<thead>
<tr>
<th>Absence of plasma</th>
<th>ΔPAP, mmHg</th>
<th>ΔW, g</th>
<th>ΔVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without COX-2 inhibitor</td>
<td>12.01 ± 0.63</td>
<td>7.60 ± 0.79</td>
<td>3.14 ± 0.42</td>
</tr>
<tr>
<td>NS-398 (10 µmol/l)</td>
<td>2.20 ± 0.20†</td>
<td>0.34 ± 0.11†</td>
<td>1.90 ± 0.40*</td>
</tr>
<tr>
<td>NS-398 (25 µmol/l)</td>
<td>0.83 ± 0.24†</td>
<td>0.15 ± 0.05†</td>
<td>1.00 ± 0.29†</td>
</tr>
<tr>
<td>SC-236 (10 µmol/l)</td>
<td>2.77 ± 0.35†</td>
<td>0.65 ± 0.11†</td>
<td>1.17 ± 0.25†</td>
</tr>
<tr>
<td>DUP-697 (25 µmol/l)</td>
<td>0.05 ± 0.03†</td>
<td>0.03 ± 0.03†</td>
<td>0.00 ± 0.00†</td>
</tr>
<tr>
<td>Presence of 1.5% plasma</td>
<td>5.64 ± 0.57</td>
<td>1.34 ± 0.33</td>
<td>2.22 ± 0.26</td>
</tr>
<tr>
<td>Without COX-2 inhibitor</td>
<td>0.14 ± 0.09†</td>
<td>0.06 ± 0.02†</td>
<td>0.00 ± 0.00†</td>
</tr>
<tr>
<td>NS-398 (10 µmol/l)</td>
<td>0.00 ± 0.00†</td>
<td>0.00 ± 0.00†</td>
<td>0.00 ± 0.00†</td>
</tr>
<tr>
<td>SC-236 (10 µmol/l)</td>
<td>0.53 ± 0.19†</td>
<td>0.00 ± 0.00*</td>
<td>0.00 ± 0.00†</td>
</tr>
<tr>
<td>DUP-697 (25 µmol/l)</td>
<td>0.33 ± 0.17†</td>
<td>0.05 ± 0.05†</td>
<td>0.00 ± 0.00†</td>
</tr>
</tbody>
</table>

Values are means ± SE of changes occurring within 5 min of AA application; n = 5 independent experiments/group. COX, cyclooxygenase. Lungs were buffer or buffer-plasma perfused in presence of 10,000 ng/ml of LPS. AA (5 µmol/l) was admixed to perfusate after a 2-h recirculation period. COX-2 inhibitors were applied 10 min before AA challenge. Significant difference from respective control lungs: *P < 0.01; †P < 0.001.

Table 3. ΔPAP, ΔW, and ΔVP in response to AA challenge: effect of anti-TNF-α neutralizing antibody

<table>
<thead>
<tr>
<th>Absence of plasma</th>
<th>ΔPAP, mmHg</th>
<th>ΔW, g</th>
<th>ΔVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, no antibody</td>
<td>12.01 ± 0.63</td>
<td>7.60 ± 0.79</td>
<td>3.14 ± 0.42</td>
</tr>
<tr>
<td>12.5 µg/l (5 min)</td>
<td>10.46 ± 1.03</td>
<td>6.24 ± 1.17</td>
<td>1.92 ± 0.32</td>
</tr>
<tr>
<td>12.5 µg/l (60 min)</td>
<td>10.30 ± 1.03</td>
<td>5.89 ± 1.90</td>
<td>2.96 ± 0.49</td>
</tr>
<tr>
<td>25 µg/l (60 min)</td>
<td>12.46 ± 1.39</td>
<td>7.92 ± 1.38</td>
<td>2.00 ± 0.19</td>
</tr>
<tr>
<td>Presence of 1.5% plasma</td>
<td>5.84 ± 0.57</td>
<td>1.34 ± 0.33</td>
<td>2.21 ± 0.26</td>
</tr>
<tr>
<td>Control, no antibody</td>
<td>4.37 ± 1.74</td>
<td>1.28 ± 0.56</td>
<td>0.97 ± 0.37*</td>
</tr>
<tr>
<td>12.5 µg/l (5 min)</td>
<td>5.70 ± 2.10</td>
<td>1.79 ± 1.02</td>
<td>0.58 ± 0.20†</td>
</tr>
<tr>
<td>25 µg/l (60 min)</td>
<td>4.16 ± 1.39</td>
<td>1.26 ± 0.52</td>
<td>0.66 ± 0.37†</td>
</tr>
</tbody>
</table>

Values are means ± SE of changes occurring within 5 min of AA application; n = 5 independent experiments/group. Lungs were buffer or buffer-plasma perfused in absence (control) and presence of 10,000 ng/ml of LPS. Anti-TNF-α neutralizing antibody was admixed to perfusate 5 min before (5 min) and 60 min after LPS application. AA (5 µmol/l) was admixed to perfusate after a 2-h recirculation period. Significant difference from respective control lungs: *P < 0.05; †P < 0.01.
increased the perfusate TxB₂ levels to ≈250 pg/ml in control lungs and ≈500–750 pg/ml in LPS-primed lungs within 5 min (Fig. 4). This AA-induced additional TxB₂ liberation was largely suppressed by NS-398 (10 µmol/l) being administered to the perfusate 10 min before AA application (Fig. 4). In addition, a more pronounced accumulation of 6-keto-PGF₁α was noted in LPS-primed lungs, amounting to 1,360 ± 6314 (1,000 ng/ml of LPS) and 1,986 ± 226 (10,000 ng/ml of LPS) pg/ml within 2 h. AA administration resulted in a further increase in perfusate PGF₁α levels to ≈2,500–3,500 ng/ml within 5 min (Fig. 4). This increase was not suppressed by 10 µmol/l of NS-398, but rather the levels of 6-keto PGF₁α were elevated in the presence of NS-398 in the LPS-primed lungs.

Although in control lungs only negligible levels of TNF-α were detected in the perfusion medium, addition of LPS caused a dose-dependent increase of TNF-α within 60–120 min (Fig. 5). As anticipated, this increase was not further enhanced by the admixture of AA to the perfusate.

Buffer-plasma-perfused lungs. The presence of 1.5% rat plasma admixed to the buffer fluid throughout the experiments did not affect the baseline values of PAP, lung weight, and VP in the control lungs. Moreover, no significant change in these variables was noted when 1,000 or 10,000 ng/ml of LPS was recirculated for 2 h in the presence of rat plasma. In contrast, a markedly more pronounced liberation of TNF-α was noted on LPS exposure in the presence of plasma constituents (Fig. 5). Concerning the responses to AA challenge, all changes in physiological variables (PAP, weight, and VP) were more moderate compared with those in the buffer-perfused lungs, but again, a marked amplification of these changes after preceding LPS incubation was noted (Table 1). In accord with the buffer-perfused lungs, all AA-elicited changes were virtually fully suppressed in the presence of the selective COX-2 inhibitors NS-398, SC-236, and DUP-697 (Table 2). In contrast to lungs perfused in the absence of plasma, the impact of the anti-TNF-α neutralizing antibodies on the AA-elicited elevation in VP was noted in buffer-plasma-perfused organs (Table 3, Fig. 3); this was significantly reduced by preapplication of the antibody either 5 min before or 60 min after LPS administration. In contrast, PAP increase and ΔW in response to AA application were not altered by prior administration of TNF-α neutralizing antibodies in the buffer-plasma-perfused lungs (Table 3).

The amounts of TxB₂ and PGF₁α, measured after 2 h of LPS priming within the buffer-plasma perfusate before and after administration of AA were comparable to those in buffer-perfused lungs.

**DISCUSSION**

In the present study, a 2-h period of LPS priming did not provoke any change in PAP, VP, and lung weight in buffer-perfused rat lungs, although enhanced baseline liberation of prostanoids and TNF-α was noted. Subsequent administration of AA did, however, provoke markedly increased PAP responses in association with
enhanced Tx release in the LPS-primed lungs. Moreover, rapid lung edema formation and an increase in VP were elicited by AA under these conditions. All changes were virtually fully suppressed by three structurally unrelated COX-2 inhibitors, whereas anti-TNF-α antibodies were ineffective. Performing the studies in the presence of rat plasma forwarded less impressive changes in the physiological variables, but corresponding efficacy of the COX-2 inhibitors was noted. Under these circumstances, the LPS-elicited TNF-α liberation was, however, markedly increased, and anti-TNF-α antibodies significantly reduced the AA-induced increase in VP.

It is well in line with previous studies (8, 36, 38, 44) in buffer-perfused rabbit and rat lungs that recirculation of substantial quantities of LPS did not provoke overt changes in functional variables such as pulmonary vascular pressure, lung weight, and VP assessed in the present investigation. The enhanced baseline liberation of TxA₂ noted during the LPS-priming period was obviously counterbalanced by increased generation of PGI₂ and possibly other vasodilators occurring under these conditions. Moreover, the accumulation of TNF-α in response to the LPS challenge apparently did not suffice to alter the physiological variables in the absence of a secondary stimulus, which is in accordance with the observation that exogenously administered TNF-α does not per se provoke vascular abnormalities in buffer-perfused lungs (6).

In contrast to the unchanged baseline variables, administration of AA after a 2-h LPS-priming period provoked a markedly increased PAP elevation, and this was clearly attributable to a “hyperreactive” COX-2-Tx axis. First, enhanced Tx liberation in response to AA administration was observed to occur in parallel with the elevated pressor response, and Tx is a well-known potent vasoconstrictor agent in the pulmonary circulation (11, 32, 35). Second, the Tx release reaction was not accompanied by a corresponding increase in PGI₂ liberation. Third, and most importantly, the pressor response was largely suppressed by three structurally unrelated selective COX-2 inhibitors (NS-398, DUP-697, and SC-236). In the concentration range currently applied, these agents were shown to be highly selective for COX-2, with no effect on COX-1 activity (30). This is even true for the higher NS-398 dose (25 µmol/l) presently employed, which virtually fully blocked any AA-elicited pressure elevation as similarly noted for 25 µmol/l of DUP-697. In addition to the recently noted fact that Tx-mediated vasoconstrictor responses proceed via COX-2 in noninflamed lungs (11), the present data thus demonstrate that this is also true for the markedly enhanced Tx and vasoconstrictor responses in lungs undergoing LPS exposure. Moreover, these findings are fully compatible with recent immunohistochemical studies in endotoxin-challenged rat lungs (12, 13), in which markedly enhanced expression of both COX-2 and thromboxane synthase was noted in the smooth muscle cells of large arteries and, in particular, of small partially muscularized arteries known to be intimately involved in the regulation of lung vascular tone (31). In contrast, COX-1 expression, predominant, e.g., in endothelial cells, was found to be fully unchanged in these investigations. Correspondingly, the COX-2 but not the COX-1 message was noted to be increased in the homogenate of lungs exposed to LPS for 2 h (8, 13). The finding that the present use of selective COX-2 inhibitors did not suppress PGI₂ formation in the LPS-primed rat lungs indeed suggests that the generation of this vasodilatory agent proceeds largely via COX-1 also under conditions of LPS exposure.

In addition to the vasoconstrictor response, AA administration provoked a moderate pulmonary edema formation in the control lungs (=1-gAW within 5 min), and this, too, was markedly enhanced in the LPS-primed lungs (=6–8 g within 5 min). Both enhanced vascular permeability and increased capillary filtration pressure might underlie this edema formation. Because partitioning of the pulmonary vascular resistance and direct assessment of lung capillary permeability were not performed in the present study, this question may not be definitely settled. However, Tx-mediated postcapillary vasoconstriction with subsequently elevated hydrostatic pressure in the capillary bed offers a most plausible explanation for this finding: 1) the edema formation occurred very rapidly after the administration of AA, whereas elevations in the capillary filtration coefficient in response to this fatty acid, mostly attributed to lipoxygenase pathways (17, 43), were commonly noted to represent a more protracted event; 2) selective COX-2 inhibition fully blocked edema formation, strongly supporting its pathogenesis via prostanoid and, in particular, Tx generation, and none of the prostanooids were hitherto found to increase directly the capillary permeability in rat lungs; and 3) Tx is known to provoke postcapillary in addition to precapillary vasoconstriction in the rat lung vasculature and may thus well be operative to increase the microvascular...
fibration pressure (19, 37). In addition to such a role for Tx in the edematous response of the LPS-primed lungs, the endotoxin-related enhanced TNF-α generation might be attributed to the readiness of the isolated organs for pressure-induced fluid accumulation because this agent was noted in vivo to increase pulmonary vascular permeability (3, 16, 25); however, such an effect was lacking in other investigations (4, 6). In the present study, TNF-α per se did not apparently suffice to provoke lung edema formation because no ΔW was noted after the 2-h LPS recirculation period and the TNF-α levels were maximally increased at this time point, with no further elevation on subsequent AA administration. Moreover, no reduction in ΔW was observed in the experiments employing anti-TNF-α neutralizing antibodies, thus largely excluding a role for this cytokine in the edematous response of the LPS-primed lungs.

In addition to the vascular abnormalities, a doubling in VP was provoked by AA in the LPS-primed lungs, which was fully lacking in the organs perfused in the absence of endotoxin. Under the presently employed conditions of volume-controlled ventilation at a preset frequency, such an increase in VP might reflect both alterations in lung compliance and changes in bronchial resistance. Random performance of static pressure-volume curves (data not shown) clearly demonstrated that increased bronchial resistance and not loss of tissue compliance is the predominant phenomenon underlying the VP elevations in the LPS-primed lungs. This is well in line with the observation that the VP increase occurred very rapidly in response to the AA admixture, even preceding edema formation (which might reduce static compliance), and the previous findings that LPS-related Tx generation provokes marked bronchoconstriction in rat lungs (40) in contrast to that in rabbit lungs (36, 38, 44). Indeed, COX-2 inhibition was presently found to inhibit significantly the VP increase in response to AA in the LPS-primed lungs. This is of interest against the background that a previous study (3) demonstrated coexpression of COX-1 and COX-2 in cultured bronchial smooth muscle cells, with COX-2 upregulation in response to LPS. In accordance with these in vitro studies, immunostaining experiments in intact rat lungs showed the presence of both COX-1 and COX-2 in bronchial epithelial cells and bronchial smooth muscle cells under baseline conditions (14), and upregulation of both COX-2 and thromboxane synthase was recently noted to occur in response to LPS in both cell types (12, 13). Thus compartmentalized Tx generation in the bronchial tissue in response to AA, occurring largely via the COX-2-Tx axis being upregulated during the period of LPS exposure and forwarding a marked bronchoconstrictor response, is the most plausible explanation for the rapid increase in VP in the endotoxin-primed lungs.

In the presence of plasma constituents, both the vasoconstrictor response and the ΔW provoked by the AA challenge were reduced, but potent inhibition of these events by all three COX-2 inhibitors was nevertheless apparent. Most probably, the lower responsiveness to AA is attributable to some protein binding of this fatty acid and/or the arising prostanoids, thus eliciting a reduced vasoconstrictor response as previously described for buffer-perfused lungs (22). This nonspecific effect of plasma proteins was apparently not “overcompensated” for by an enhanced priming potency of LPS in the presence of specialized plasma constituents, among which LBP and soluble CD14 may be of major interest (15, 34, 39, 41, 45). It is in line with such reasoning that a recent immunohistochemical study (13) showed LPS-induced COX-2 upregulation in smooth muscle cells of partially muscularized vessels to occur independently of plasma components. In contrast, markedly enhanced TNF-α generation was noted in the presence of plasma components, well compatible with a large body of in vitro studies (23, 24, 29, 46) demonstrating LBP dependency of the cytokine release reaction in different types of leukocytes and a previous investigation (44) in perfused rabbit lungs in which TNF-α generation in both the intravascular and bronchoalveolar compartments was noted to be manyfold increased in the presence of small quantities of plasma proteins. Cytokines, and in particular TNF-α, are known to elicit regulatory events in a large number of cell types, and they have also been implicated in the upregulation of COX-2 (1, 2). The current employment of anti-TNF-α neutralizing antibodies applied at concentrations sufficient to bind the total amount of perfusate TNF-α did not, however, support the view that the LPS-elicited upregulation of COX-2 in the vascular smooth muscle cells, assumed to underlie the enhanced Tx-elicited vasoconstrictor response as detailed above, might be mediated in an indirect fashion via liberation of this cytokine in both the absence and presence of plasma constituents. In contrast, these experiments forwarded evidence for a role of TNF-α in the bronchial compartment (the priming of the bronchoconstrictor response under conditions of plasma admixture), and thus high levels of TNF-α generation were markedly suppressed by anti-TNF-α antibodies. This finding thus supports the concept that proinflammatory cytokines like TNF-α may be involved in the pathogenesis of airway hyperreactivity as suggested from models of asthma pathogenesis (42, 47). It may not be fully settled by the current data whether this effect of TNF-α proceeded largely via upregulation of COX-2 because selective COX-2 inhibitors were invariably effective in suppressing the increase in VP under these conditions or whether elicitation of additional bronchoconstrictor agents might be involved as, e.g., suggested for endothelin (42, 47).

In conclusion, LPS exposure of rat lungs profoundly altered the readiness of these organs to respond to a subsequent AA challenge. Enhanced Tx generation occurring via upregulation of COX-2, in particular in vascular smooth muscle cells, is suggested to underlie an increased vasoconstrictor response and rapid lung edema formation, and these events occur independent of plasma constituents and LPS-induced TNF-α generation. In addition, COX-2-dependent bronchoconstriction is noted in the LPS-primed lungs, with high levels of TNF-α arising in the presence of endotoxin plus...
plasma components contributing to this phenomenon. In agreement with a recent immunohistological study in LPS-primed lungs (14), the present pharmacological data support the view of a concordant induction of COX-2 and terminal thromboxane synthase in both the vascular and bronchial smooth muscle cells as the predominant event underlying the enhanced pulmonary readiness to respond to a secondary stimulus after preceding endotoxin exposure.

We thank G. Müller for excellent technical assistance. We are grateful to Dr. R. L. Snipes (Department of Anatomy, J ustus-Liebig-University Giessen, Giessen, Germany) for linguistically reviewing the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft SFB 547 (Kardiopulmonales Gefässsystem). This publication includes parts of the thesis of M. Merkle in partial fulfillment for the MD degree. Address for reprint requests and other correspondence: L. Ermert, Institut fuer Anatomie und Zellbiologie, Aulweg 123, 35385 Giessen, Germany (E-mail: leander.ermert@anatomie.med.uni-giessen.de).

Received 27 September 1999; accepted in final form 19 January 2000.

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